Further Researches on the Development of Trypanosoma gambiense *in* Glossina palpalis.

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The object of these experiments was to try to discover if there is any definite cycle of development of the trypanosome of Sleeping Sickness in the tsetse fly, *Glossina palpalis*, and if the late or renewed infectivity of the fly coincides with any phase in this development.

The mode of experimentation was to feed a cageful of *laboratory-bred* tsetse flies on an animal whose blood contained numerous trypanosomes, and at the end of various times to kill the flies and examine their intestinal contents. This was done for periods of one day, two days, three days, and so on, up to 56 days. The microscopical examination of preparations made from the intestinal contents on the various days gave information as to the number and appearance of the trypanosomes.

After the infective feed or feeds the flies were fed every day on a healthy animal, so that by the appearance of trypanosomes in the animal's blood the day on which one or more of the flies became infective could be arrived at.

Method Used in the Examination of the Flies.

The flies were killed by exposing them to the vapour of chloroform. After being killed the proboscis and pharynx were removed and examined under a cover-glass with the high and low powers. The terminal segment of the abdomen of the fly was then snipped off, and the whole abdominal viscera gently pressed out. This was moistened with a little normal saline solution, and the gut unravelled without rupturing. The proventriculus and crop were often pulled out intact with the gut. The whole thoracic and abdominal tract could then be laid out in line and examined under a low power.

In taking out the gut it was generally possible to draw out with it the abdominal portion of the salivary glands, which could then be separated without contamination from accidental rupture of the gut. If the salivary glands or proventriculus remained behind they were dissected out after removal of the gut. In every case these organs were thoroughly washed in

three changes of normal saline solution, in order to minimise the chance of their being contaminated by accidental rupture of the intestines.

The stained specimens were examined day after day, and coloured drawings, at a magnification of 2000 diameters, made of all the different forms met with. The drawings of the trypanosomes found in the proboscis, proventriculus, fore-gut, mid-gut, hind-gut, proctodæum, and salivary glands, were kept separate, so that a series of drawings of trypanosomes taken from any one part, from the first day of infection to the 56th day, could be compared.

By arranging these drawings on a wall the horizontal layers would represent the contents of, say, the fore-gut from one day onwards, while the vertical rows would represent the trypanosomes found in the whole length of a fly for one day. More than six hundred drawings were made in this way, so that it seems impossible that any important form can have been left out.

General Considerations regarding the Development of the Trypanosomes in the Fly.

Let us now take a general view of the types of trypanosomes found in the various parts. It is evident that very few of the six hundred drawings can be reproduced; a few types, taken here and there, must suffice. For the first three or four days trypanosomes are found in all the flies, but at the end of six or seven the trypanosomes have disappeared out of many of them. That is to say, it is only in a certain percentage that further development takes place. In one series this was 8 per cent. In 92 per cent., then, of flies which imbibe infected blood, the trypanosomes simply degenerate and die out within the first few days. In 8 per cent., on the other hand, the trypanosomes find conditions more favourable for development, and increase to a marvellous extent, filling the whole of the fore-gut, mid-gut, and hind-gut with countless swarms of trypanosomes.

How long this development continues is unknown. It is considered probable that it continues for the remainder of the fly's life, and this has been proved by experiment up to 96 days.

What the conditions in the intestine of the fly are, which render this development possible, are also unknown. It was thought that it might depend on the reaction of the intestinal fluids. This is, however, found on examination to be faintly acid in all flies, whether development has taken place or not. The presence of bacteria also seems to have no connection with the phenomenon. Sex, moreover, has no influence; development occurs in as many males as females.

TYPES OF TRYPANOSOMA GAMBIENSE FOUND IN THE ALIMENTARY CANAL.

It would serve no good purpose to describe separately, day by day, the types found in the various parts of the alimentary canal, as they run into each other in such a way that any classification of them seems impossible.

The following table represents, approximately, the numbers found in the different parts of the alimentary canal at various times after infection. The — sign means that an examination was made and nothing found. ± means few. +, many. + +, very many. + + +, swarming. If no sign, then no examination has been made. (See p. 516.)

The Proboscis.—In our experience *Trypanosoma gambiense* is never found in the proboscis of *Glossina palpalis*, except immediately after an infected feed, when for a short time blood containing trypanosomes may be seen in the lumen of the proboscis. This is very different from what obtains in an infection by *Trypanosoma vivax*, in which case the proboscis is alone infected.

Proventriculus.—As seen from the above table, this part of the alimentary canal is sometimes found empty when the remainder of the gut is swarming.

Fore-, Mid-, and Hind-gut.—It is here that the greatest development of the trypanosomes is found. Among the extraordinary numbers and diversity of type it is difficult or impossible to find one's way. Generally speaking, the trypanosomes found during the first few days are merely degenerating blood forms. After this there appears a type of trypanosome which remains dominant throughout the whole developmental period. This is a long, moderately broad form, the protoplasm staining well, without granules or vacuoles, having an oval compact nucleus situated in the centre of the body, a small round micronucleus lying at some distance from the elongated snout-like posterior extremity, the undulating membrane narrow and simple, and the flagellum proceeding little, if anything, beyond the protoplasm of the cell. The flagellum also appears very frequently to arise from a pink-coloured body situated near the micronucleus, an appearance never seen in the normal blood trypanosomes.

This seems to be the healthy normal developing type in the intestine of the fly. It is seen in all parts of the intestine and at all times. It forms masses of innumerable individuals alike in size and shape. When a fresh supply of blood is taken in by the fly this type can be imagined to multiply with extraordinary rapidity. When the blood supply runs low then this type can also be imagined as degenerating and disappearing just as rapidly. The host of diverse forms which thus arises beggars description. Some are round or oval in shape, 3 or 4 microns in diameter, with or without a flagellum. From this simple form all shapes and sizes can be seen up to the huge shapeless mass of protoplasm, multi-nucleated and multi-flagellated.

Table I.—Number of Trypanosomes found in the Various Parts of the Alimentary Canal and Salivary Glands.

Time, days.	Pro-boscis.	Proventri-culus.	Crop.	General smear of gut.	Fore-gut.	Mid-gut.	Hind-gut.	Procto-dæum.	Salivary glands.
1	–	–				+ +			–
1	–	–				+	+		–
2	–				+	+	+ +		–
3	–					+ +	+ +		
4	–				+	+	+	–	–
5	–	–	–		–	–	–		–
6	–	–	–	+	+	+			–
7	–	–	–	+					–
8	–	+			+	+ +	+ +		–
8	–				±	±	±		
9	–	–			±	+ +		–	–
9	–				+	+	+		
10	–				+	+ +	+ +		
10	–					+			
11	–	±	–	+ +	–	±	±		–
11	–	±	–	+ +	+ +	±			–
11	–	+ +			+ +	+ +	+ +		–
14	–	+ + +	–		+ +	+ +		–	–
14	–	+ +	–		+ +	+ +	+ +		–
15	–	–			+	+			–
17	–	–	–		±	+ +	+ +		–
17	–	+ +		–	+ +	+ +			–
18	–	–			+ +	+	+ +		–
18	–					+ +	+ +		–
20	–	+ +			+ +	+ +	+ +	±	–
21	–			+ + +					
22	–			+ +					
23	–				+	+	+		
24	–				+ + +	+ + +	+ + +		
25	–	–	–		+	+ + +	+ +		+
28	–			+ +		+ +			+
30	–	+			+ +	+ +			+
30	–	+			+ +	+ +	±		–
31	–	+	+		+ + +	+ +			–
31	–			+					
34	–			+ +	+ +	+ + +	+ + +	–	–
35	–	–			+	+ +	+ +		–
36	–			+ + +	+ + +	+ + +	+		+
36	–	–		+ + +	+ +	+ + +			–
36	–	+ +		+ + +					
37	–			+ +					
40	–			+ +					
40	–			+ +					
42	–	+ +		+	+ +				+
43	–			+ +					+ +
44	–	+			+ +	+ + +			+
44	–				+	+ +	+ +		+
44	–	+			+	+	+		+
46	–	+			+ + +	+ + +	+ + +		+ +
49	–	–	–		+	±			–
51	–		–		+	+	+ +		–
53	–	–			±	±			–
53	–	–			+	+ +			+
53	–		–			±			–
56	–			+ +					+

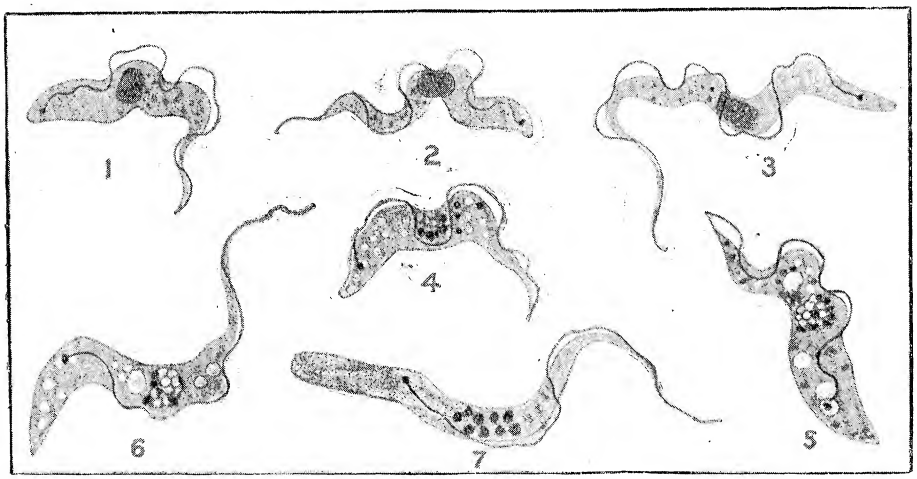
In our experience, the long narrow forms, described by some observers as "male" forms, are exceedingly rare, and it seems impossible to ascribe to to them any very important *rôle* in the process of development. Crithidia-like forms are also exceedingly rare—that is, forms with the micronucleus close to or anterior to the nucleus.

Types of Trypanosoma gambiense *found in the Salivary Glands.*—On referring to Table I it will be seen that trypanosomes did not appear in these glands until the twenty-fifth day, but that after this time they were usually present. In our opinion, this invasion of the salivary glands is of the greatest importance in the history of the development of *Trypanosoma gambiense* in *Glossina palpalis*. Here it may be parenthetically remarked that, because one kind of development takes place with one species of trypanosome and one species of tsetse fly, it by no means follows that the same thing will occur either with another species of trypanosome or another species of fly. Each combination must be worked out separately and nothing left to analogy. *Trypanosoma vivax* and *Glossina palpalis* afford a striking example of this.

In the development of *Trypanosoma gambiense* in *Glossina palpalis* one circumstance, which we think of prime importance, emerges, and that is, that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal blood-type. It must not be imagined, however, that the salivary glands show no other forms but this blood-type. On the contrary, there are many other forms seen; but here only are found trypanosomes apparently identical with the short and stumpy forms found in the blood. What causes or leads up to this reversion to the blood-type in the salivary glands is quite unknown, but, as will be seen later, the *Glossina palpalis* does not become infective by biting until this invasion of the salivary glands takes place.

How the trypanosomes find their way into the salivary glands is also quite unknown. It seems highly improbable that they pass from the alimentary canal by way of the salivary duct, and as they are never found in the body-cavity, it is also difficult to see how they can make their way directly from the intestine to the abdominal portion of the salivary glands.

ILLUSTRATIONS OF VARIOUS MODIFICATIONS IN SHAPE OF TRYPANOSOMA GAMBIENSE IN GLOSSINA PALPALIS.



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FIGS. 1—3.—Normal blood parasites (*Trypanosoma gambiense*).
FIG. 4.—24 hours after ingestion by the fly.
FIGS. 5 and 6.—48 hours after ingestion by the fly.
FIG. 7.—96 hours after ingestion by the fly.

Figs. 1—7 represent the trypanosomes as they appear in the intestine of *Glossina palpalis* during the first few days. Figs. 1—3 are ordinary blood forms, as seen immediately after the fly has fed, and before any change has taken place. Figs. 4—7 represent the process of degeneration which takes place during the first four days. The body swells up, the nucleus breaks up, and the cytoplasm becomes vacuolated.

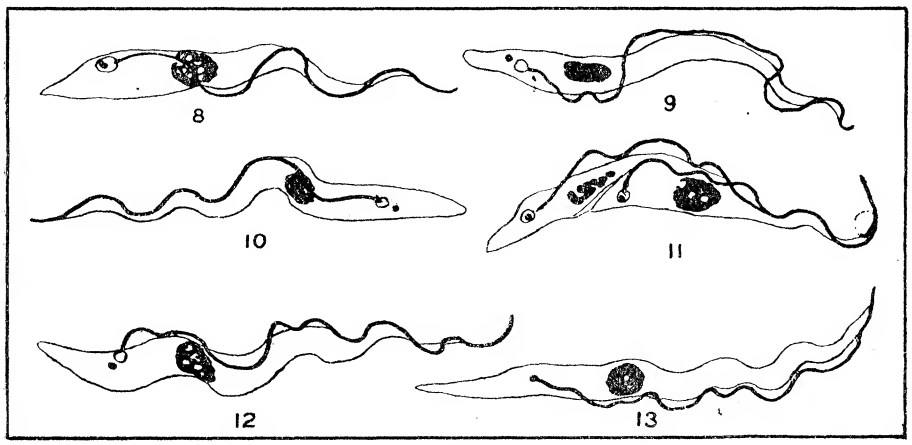

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FIG. 8.—*Trypanosoma gambiense* from fore-gut, 8 days after infected feed.
FIG. 9.—*T. gambiense* from proventriculus, 14 days after infected feed.
FIG. 10.—*T. gambiense* from fore-gut, 18 days after infected feed.
FIG. 11.—*T. gambiense* from mid-gut, 25 days after infected feed.
FIG. 12.—*T. gambiense* from mid-gut, 34 days after infected feed.
FIG. 13.—*T. gambiense* from fore-gut, 44 days after infected feed.

Figs. 8—13 represent what we consider to be the normal reproductive or developing type found throughout the intestine during the whole period of development. It is to be noted that this form is longer and broader than the normal broad form; the protoplasm is clear, and stains readily and evenly, and this cell looks normal. The nucleus is compact and situated nearer the posterior extremity than the anterior. The micronucleus is small and round, lying at some distance from the elongated posterior extremity. Many dividing forms of this type can be seen. In our opinion, this is the common multiplying form, and from it arises an infinite variety of degenerating forms.

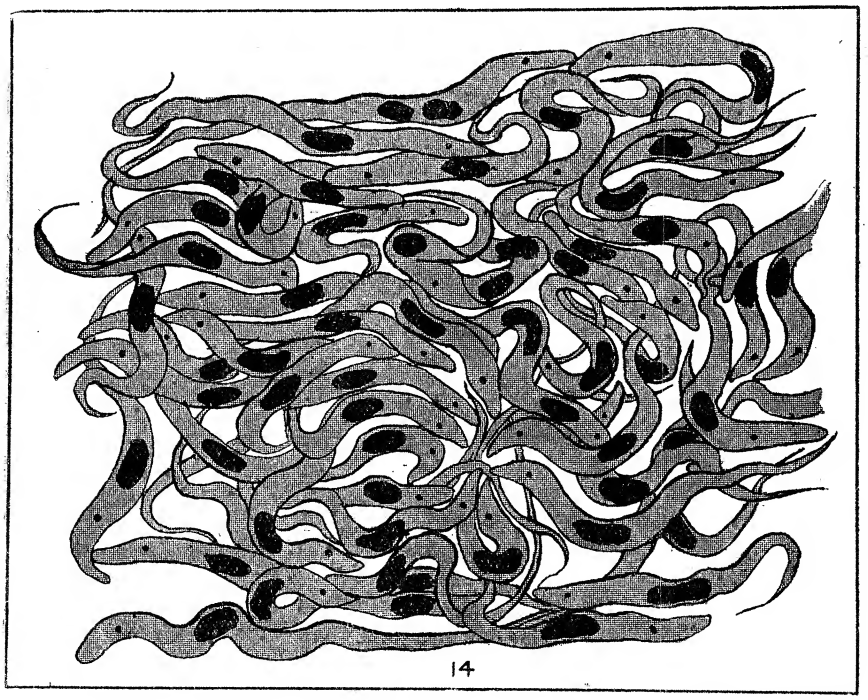

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Fig. 14.—Part of a mass of *Trypanosoma gambiense* from the mid-gut.

This figure is given in order to try to convey some idea of the enormous proliferation of trypanosomes which takes place in the intestine of *Glossina palpalis*. Throughout the whole length of the alimentary canal, from the proventriculus to the proctodæum, this condition is frequently seen, and in the living condition presents an extraordinary spectacle. Looked at through the wall of the intestine the matted masses are seen in active movement, swaying about and wriggling in every direction. They do not appear to be attached to the wall of the intestine, but move about freely, and when the intestinal wall is burst pour out in countless numbers. The trypanosomes evidently belong to the type figured in the preceding sketch, called the normal reproductive type.

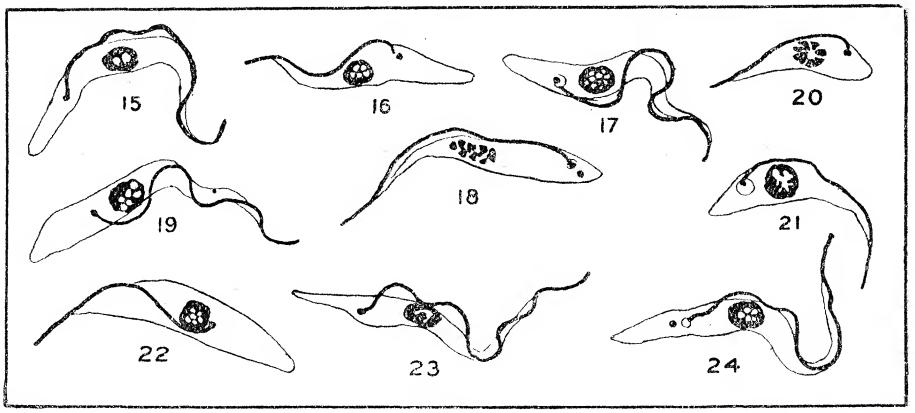

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Fig. 15.—*Trypanosoma gambiense* from hind-gut, 8 days after infected feed.
Figs. 16, 17, and 18.—*T. gambiense* from mid-gut, 14, 20, and 30 days after infected feed.
Fig. 19.—*T. gambiense* from hind-gut, 34 days after infected feed.
Figs. 20 and 21.—*T. gambiense* from proventriculus, 36 days after infected feed.
Fig. 22.—*T. gambiense* from hind-gut, 44 days after infected feed.
Figs. 23 and 24.—*T. gambiense* from mid-gut, 46 and 53 days after infected feed.

Figs. 15—24 represent smaller forms which are fairly common and occur throughout the intestine and at all times. The examples given above are taken from the eighth day to the fifty-third day. They are called by us "small developmental forms," since they resemble the larger in having clear protoplasm and a compact nucleus. Dividing forms are often seen.

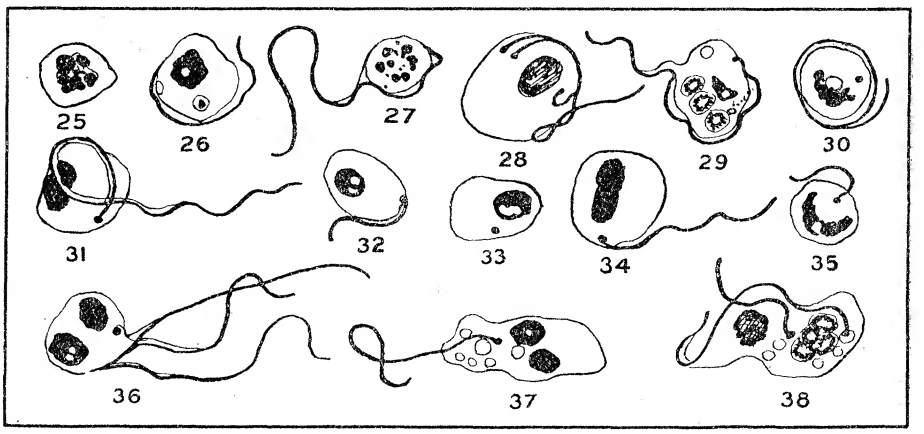

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Fig. 25.—*Trypanosoma gambiense* from mid-gut, 8 days after infected feed.
Fig. 26.—*T. gambiense* from fore-gut, 11 days after infected feed.
Figs. 27 and 28.—*T. gambiense* from mid-gut, 16 and 17 days after infected feed.
Figs. 29, 30, and 31.—*T. gambiense* from fore-gut, 17 and 18 days after infected feed.
Fig. 32.—*T. gambiense* from hind-gut, 20 days after infected feed.
Figs. 33 and 34.—*T. gambiense* from fore-gut, 24 days after infected feed.
Figs. 35, 36, and 37.—*T. gambiense* from mid-gut, 24, 44, and 46 days after infected feed.
Fig. 38.—*T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 25—38 represent round and irregularly-shaped forms of the parasite, taken from the eighth to the forty-sixth day of development. It is impossible to say what is exactly the origin of these forms—whether by the segmentation of large masses, or simply by the division and subdivision of irregular forms. Some of the examples figured are evidently dividing, as they show several nuclei and flagella. Whether the round aflagellar forms correspond to the so-called "latent" forms of various writers it is impossible to say. Those possessing flagella were active during life. In our opinion, they may be looked upon as part of the degenerative processes which are constantly taking place in the intestine of the fly.

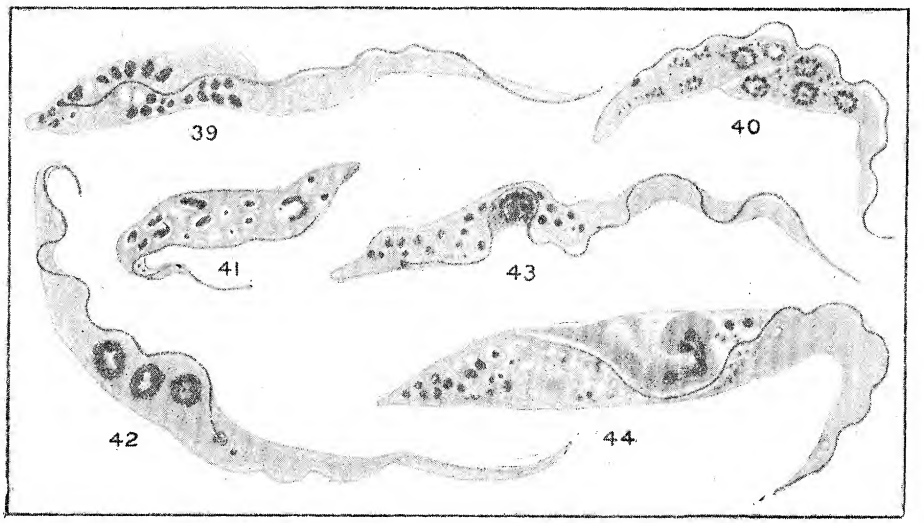

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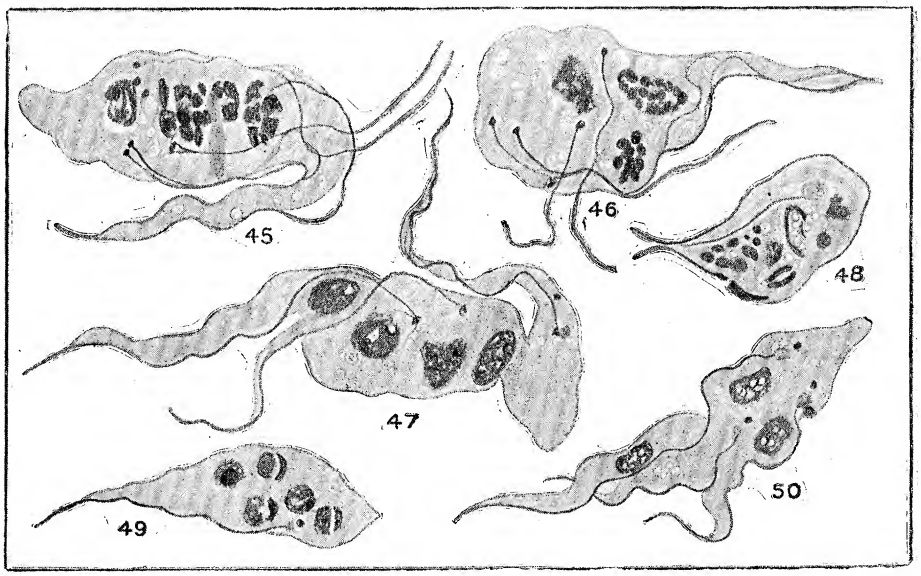
Fig. 39.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 40.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 41.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 42.—*T. gambiense* from hind-gut, 34 days after infected feed.
Fig. 43.—*T. gambiense* from mid-gut, 34 days after infected feed.
Fig. 44.—*T. gambiense* from hind-gut, 46 days after infected feed.

Figs. 39—44 represent what appear to us to be degenerative forms of the "normal reproductive type." They are of all sizes and shapes, and the cell-contents are scattered over with broken-up nuclei, or at least granules of some stainable substance. Figs. 39, 43, and 44 are also vacuolated.



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Figs. 45, 46, and 47.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 48.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 49.—*T. gambiense* from proventriculus, 30 days after infected feed.
Fig. 50.—*T. gambiense* from mid-gut, 46 days after infected feed.

Figs. 45—50 represent some of the more exaggerated types of degenerative forms. As will be seen from the drawings, they are huge, mis-shaped masses of protoplasm, multi-nucleated and, as a rule, multi-flagellated.

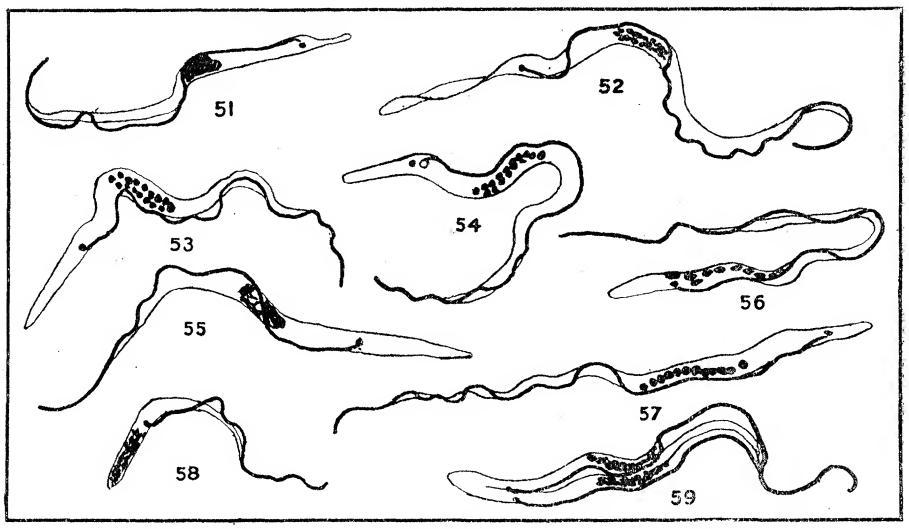

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Fig. 51.—Slender type of *Trypanosoma gambiense* from proventriculus, 17 days after infected feed.

Fig. 52.—Slender type of *T. gambiense* from mid-gut, 17 days after infected feed.

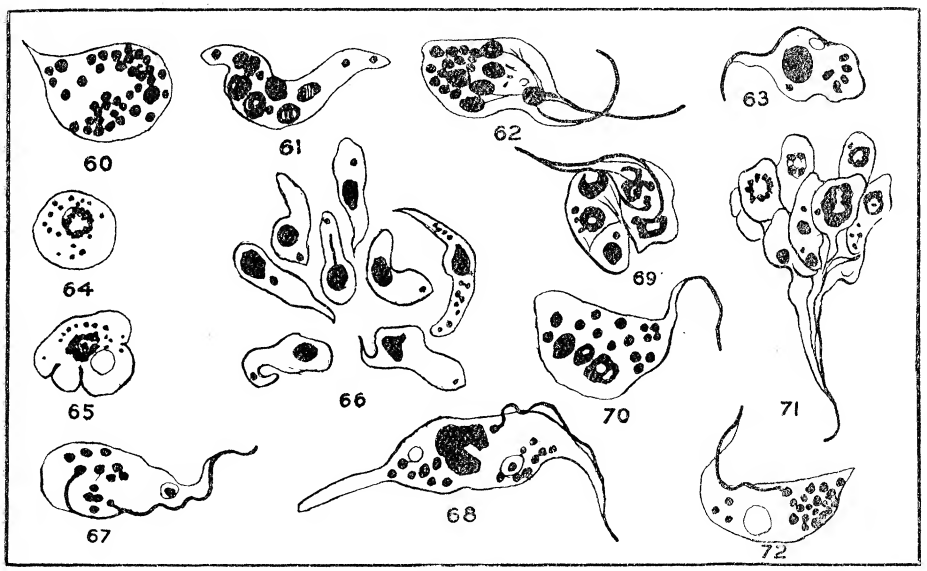
Fig. 53.—Slender type of *T. gambiense* from hind-gut, 20 days after infected feed.

Figs. 54 and 55.—Slender types of *T. gambiense* from fore-gut, 24 and 30 days after infected feed.

Figs. 56 and 57.—Slender types of *T. gambiense* from proventriculus, 44 days after infected feed.

Figs. 58 and 59.—Slender types of *T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 51—59 represent various varieties of the slender type of *Trypanosoma gambiense* found in the intestine of *Glossina palpalis*. Fig. 59 is dividing. Fig. 58 is a Crithidia type. These slender and crithidial types are uncommon, and no special connection between them and the onset of infectivity in the fly has been made out.



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Figs. 60 and 61.—*Trypanosoma gambiense* from salivary glands, 25 days after infected feed.

Fig. 62.—*T. gambiense* from salivary glands, 30 days after infected feed.

Fig. 63.—*T. gambiense* from salivary glands, 42 days after infected feed.

Figs. 64, 65 and 66.—*T. gambiense* from salivary glands, 44 days after infected feed.

Figs. 67 and 68.—*T. gambiense* from salivary glands, 46 days after infected feed.

Figs. 69 and 70.—*T. gambiense* from salivary glands, 53 days after infected feed.

Figs. 71 and 72.—*T. gambiense* from salivary glands, 56 days after infected feed.

Figs. 60—72 represent aberrant forms of *Trypanosoma gambiense* seen in the salivary glands. The first thing to be noted is the fact that the trypanosomes do not appear in the salivary glands until a late date ; in this case the earliest appearance is 25 days after the infected feed. How they reach these glands is up to the present unknown. In what shape they reach the glands is also unknown. Figs. 64, 65, 69 and 71 might be said to point to some process of segmentation, of which the finished product is represented by fig. 66.

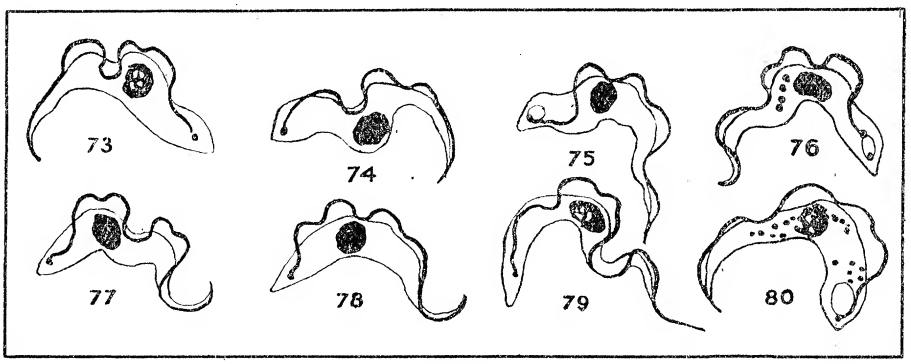

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Fig. 73.—*Trypanosoma gambiense* from salivary glands, 34 days after infected feed.
Fig. 74.—*T. gambiense* from salivary glands, 42 days after infected feed.
Fig. 75.—*T. gambiense* from salivary glands, 43 days after infected feed.
Figs. 76, 77, and 78.—*T. gambiense* from salivary glands, 46 days after infected feed.
Figs. 79 and 80.—*T. gambiense* from salivary glands, 56 days after infected feed.

When alluding, generally, in a previous part of this paper to the types of *Trypanosoma gambiense* found in the salivary glands, it was said that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal type found in the blood. Figs. 73—80 illustrate this reversion. By comparing them with figs. 1, 2, and 3, which represent normal blood trypanosomes, it will be seen that they are very similar to the short and stumpy form found in the blood. No such forms have ever been seen in any other part of the fly, and we would suggest that the occurrence of these forms in the salivary glands, coinciding as it does with the renewed infectivity of the fly, is more than mere coincidence.

Infectivity of Trypanosoma gambiense after its Development in Glossina palpalis, as shown by the Bites of the Flies giving rise to the Disease in Healthy Animals.

As has been shown in a previous paper,* the fly ceases to be infective by biting within a short time of its infective feed. From this time on for some 28 days the fly remains non-infective. Table II (p. 526) illustrates this.

From that Table it will be seen that in this series of experiments the flies first became infective 28 days after the infective feed, and that after this time the flies are usually found to be capable of giving rise to the disease by their bites.

It was stated above that the most important discovery made in this research is the connection between the invasion of the salivary glands and the infectivity of the fly. That this appears to be so is shown in Table III.

* 'Roy. Soc. Proc.,' B, 1911, vol. 82, p. 498.

Table II.—The Result of the Bites of Flies at Varying Periods after an Infective Feed.

No. of days after infective feed.	Result of bites.	No. of days after infective feed.	Result of bites.
1	—	28	+
2	—	30	—
3	—	31	—
4	—	34	+
5	—	35	—
6	—	36	—
8	—	37	+
9	—	40	+
11	—	40	+
14	—	42	+
15	—	43	+
17	—	44	+
18	—	46	+
20	—	51	—
23	—	53	+
25	—	56	+

Table III.—To show Correlation between the Invasion of the Salivary Glands of *Glossina palpalis* by *Trypanosoma gambiense* and Infection by the Bite of the Fly.

Experiment No.	No of days after infective feed.	Bites of fly infective or non-infective.	Salivary glands.	Remarks.
1910	1	—	—	
1910	2	—	—	
1910	3	—	—	
1910	4	—	—	
1910	5	—	—	
1910	6	—	—	
1910	7	—	—	
1894	8	—	—	
1894	9	—	—	
2216	10	—	—	
1894	11	—	—	
1871	14	—	—	
1693	15	—	—	
1945	17	—	—	
1945	18	—	—	
1945	20	—	—	
1718	25	—	+	Blood-type not present.
1602	28	+	+	Blood-type present.
1801	30	—	—	
1945	31	—	—	
1760	34	+	+	,, ,,
1769	36	—	—	
1712	42	+	+	,, ,,
2034	43	+	+	,, ,,
1549	44	+	+	,, ,,
2034	44	+	+	,, ,,
1706	51	—	—	
1566	53	+	+	,, ,,
1651	56	+	+	,, ,,

From the above table it is seen that the salivary glands first become invaded 25 days after the infecting feed. In this case the monkey did not become infected. This may have been due to an accident, such as this particular fly not biting the monkey, or the invasion of the salivary glands only taking place after the fly had bitten, or to the fact that the blood-type of trypanosome was not present. With this exception all the other experiments, 12 in number, confirm the hypothesis that the fly does not become infective until the salivary glands are invaded.

Another point which comes out with striking clearness is, that in all the salivary glands from flies which gave a positive result, trypanosomes similar to the short and stumpy blood-type were invariably present.

It is to be hoped that before long the result of this work will be confirmed, and so added to, that the whole story of the development of the trypanosomes within the fly, and their passage into the salivary glands, will be unfolded.

Conclusions.

1. In the course of the development of *Trypanosoma gambiense* in *Glossina palpalis* the proboscis does not become involved, as in the case of some other species.

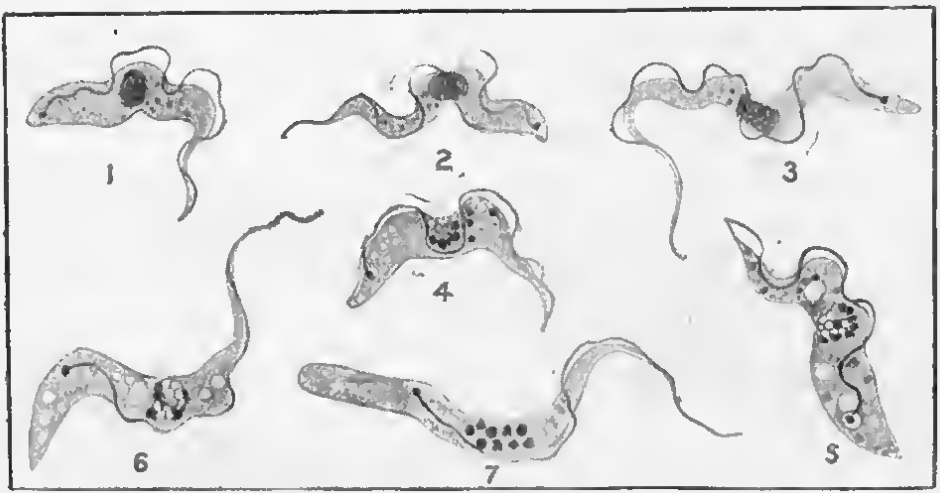
2. A few days after an infective feed the trypanosomes disappear out of the great majority of the flies, but in a small percentage this initial disappearance is followed by a renewed development.

3. After a very short time the flies which have fed on an infected animal become incapable of conveying the infection by their bites, and this non-infectivity lasts for some 28 days, when a renewed or late infectivity takes place.

4. A fly in which this renewed or late infectivity occurs can remain infective for at least 96 days.

5. An invasion of the salivary glands occurs at the same time as this renewal of infectivity, and without this invasion of the salivary glands there can be no infectivity.

6. The type of trypanosome found in the salivary glands when the fly becomes infective is similar to the short stumpy form found in vertebrate blood, and it is believed that this reversion to the blood-type is a *sine quâ non* in the infective process.



× 2000.

Figs. 1—3.—Normal blood parasites (*Trypanosoma gambiense*).
Fig. 4.—24 hours after ingestion by the fly.
Figs. 5 and 6.—48 hours after ingestion by the fly.
Fig. 7.—96 hours after ingestion by the fly.

Figs. 1—7 represent the trypanosomes as they appear in the intestine of *Glossina palpalis* during the first few days. Figs. 1—3 are ordinary blood forms, as seen immediately after the fly has fed, and before any change has taken place. Figs. 4—7 represent the process of degeneration which takes place during the first four days. The body swells up, the nucleus breaks up, and the cytoplasm becomes vacuolated.

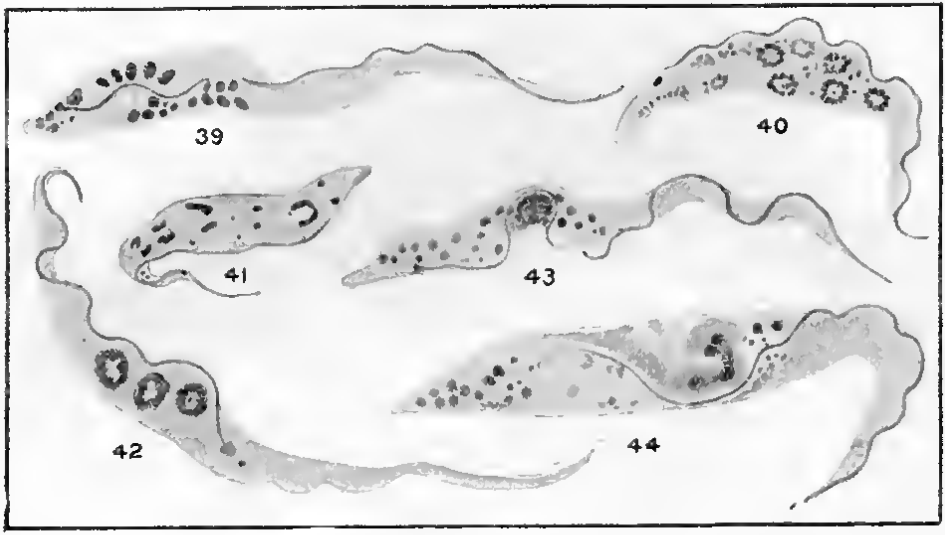


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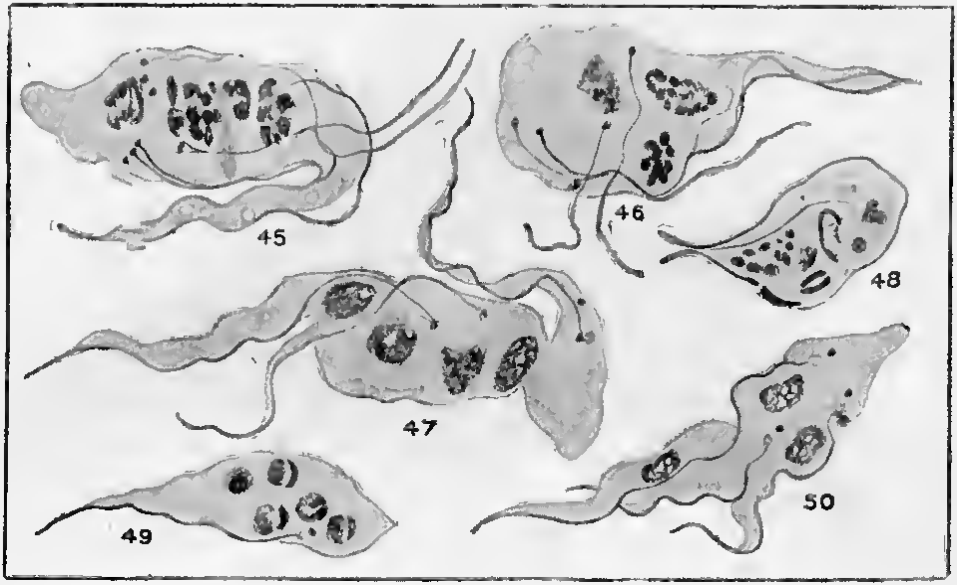
Fig. 39.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 40.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 41.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 42.—*T. gambiense* from hind-gut, 34 days after infected feed.
Fig. 43.—*T. gambiense* from mid-gut, 34 days after infected feed.
Fig. 44.—*T. gambiense* from hind-gut, 46 days after infected feed.

Figs. 39—44 represent what appear to us to be degenerative forms of the "normal reproductive type." They are of all sizes and shapes, and the cell-contents are scattered over with broken-up nuclei, or at least granules of some stainable substance. Figs. 39, 43, and 44 are also vacuolated.



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Figs. 45, 46, and 47.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 48.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 49.—*T. gambiense* from proventriculus, 30 days after infected feed.
Fig. 50.—*T. gambiense* from mid-gut, 46 days after infected feed.

Figs. 45—50 represent some of the more exaggerated types of degenerative forms. As will be seen from the drawings, they are huge, mis-shaped masses of protoplasm, multi-nucleated and, as a rule, multi-flagellated.